

Application No. 10/038,279

Paper Dated: November 10, 2003

In Reply to USPTO Correspondence of September 9, 2003

Attorney Docket No. 4029-012233

AMENDMENTS TO THE CLAIMS

1. (Original): A medium of cell culture reagents, for the maintenance and growth of a pluripotent and/or germ line competent mammalian embryonic (ES) stem cell line, which medium is conditioned by a fibroblast cell clone that produces leukemia inhibitory factor.

2. (Original): The medium of claim 1, wherein the LIF producing fibroblast cell clone further comprises immortalized rabbit fibroblasts, and further comprises an animal serum or an animal serum replacement.

3. (Original): The medium of claim 1, wherein the cell culture reagents are selected from the group consisting of inorganic salts, amino acids, vitamins and sugars.

4. (Original): The medium of claim 2, wherein the serum is a fetal animal serum.

5. (Original): The medium of claim 2, wherein the serum is a newborn animal serum.

6. (Original): The medium of claim 1, comprising reagents selected from the group consisting of Phosphate Buffered Saline (PBS); Dulbecco's Modified Eagle Media (D-MEM); Iscove's Modified Media; Dulbecco's Media; McCoy's SA Media; Minimum Essential Media Eagle (MEM); RPMI Media 1640; Medium 199; MCDB Medium; RPMI; Glasgow Minimum Essential Media (GMEM); DMEM/F-12 Media; Hams F-10 Nutrient Mixture; Lebovitz's L-15 Media; CMRL Media; BGJb Medium; Basal Medium Eagle (BME); Brinster's BMOC-3 Medium; Williams Media E; and McCoy's Media.

7. (Original): The medium of claim 4, wherein the fetal animal serum is fetal bovine serum (FBS).

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8. (Original): The medium of claim 7, wherein the fetal bovine serum is treated by a treatment selected from the group of dialysis, gamma irradiation or heat inactivation.

9. (Original): The medium of claim 1, further comprising a reducing agent.

10. (Original): The medium of claim 9, wherein the reducing agent is selected from the group consisting of 2-mercaptoethanol and microhydrin.

11. (Original): The medium of claim 1, further comprising an antibiotic.

12. (Original): The medium of claim 11, wherein the antibiotic is selected from the group consisting of penicillin, streptomycin and gentamycin.

13. (Original): The medium of claim 1, further comprising L-glutamine.

14. (Original): The medium of claim 1, further comprising EGTA.

15. (Original): The medium of claim 1, further comprising albumin.

16. (Original): The medium of claim 4, wherein the serum is derived from an animal selected from the group consisting of sheep, pigs, chickens and horses.

17. (Original): The composition of claim 16, wherein the immortalized fibroblasts have been transfected, transformed or infected by a vector overexpressing a LIF gene.

18. (Original): The composition of claim 17, wherein the LIF gene is a rabbit LIF gene.

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19. (Orginal): The composition of claim 18, wherein the fibroblast cell line used for conditioning is the Rab9 #19 cell line, which has been deposited with the Belgian Coordinated Collection of Microorganisms, under accession number LMBP 5479 CB.

20. (Original): A process of culturing mammalian ES stem cells to obtain pluripotent and/or germ line competent ES cells, wherein the culturing of the mammalian ES stem cells is at least partially performed in a composition as claimed in claim 1.

21. (Original): The process of claim 20, comprising the steps of: a) culturing cells of blastocyst stage embryos; b) culturing isolated inner mass cells; and c) passaging the inner mass cells periodically in a composition as claimed in claim 1.

22. (Original): The process of claim 21, wherein the inner mass cells are periodically passaged for at least eight times.

23. (Original): The process of claim 20, further comprising the step of producing transgenic animals.

Claims 24-38 (Cancelled)

39. (New): Embryonic stem (ES) cell line with germ line transmission capability obtained by the process of claim 20.

40. (New): The cell line according to claim 39, which has germ line transmission capability after 11 or more passages.

41. (New): The cell line of claim 39, wherein the cell line is a murine cell line.

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42. (New): The cell line of claim 41 wherein the cell line has been derived from cells or tissues with 129/SvEv, C57BL/6N; C57BL/6J-HPRT; BALB/c; CBA/CaO1a; 129/SvJ; DBA/2n; DBA/1 Ola; C3H/HeN; C57B1; 6Jola; FVB or Swiss Webster genetic backgrounds.

43. (New): The cell line of claim 42, which has germ line transmission capability after 11 or more passages.

44. (New): Embryonic stem (ES) cell line of claim 39, characterised by three-dimensional colony formation, positive staining for alkaline phosphatase; and negative staining for cytokeratin 18 and vimentin after more than 10 passages.

45. (New): Embryonic stem (ES) cell line of claim 39, for use in the generation of chimeric or ES cell derived animals.

46. (New): Embryonic stem cell line of claim 39 altered by homologous or non-homologous recombination.

47. (New): Embryonic stem cell line of claim 39, for use in the generation of animals with gene alteration via germ line transmission.